During the inhalation of ethanol, treated mice showed locomotor depression and ataxia. They also ate and drank less than control mice. These differences were not large, and treated mice showed little change in weight compared to controls. The death rate of treated mice was between 10 and 15%.

It was necessary to increase the concentration of ethanol in the inspired air throughout the experiment in order to maintain the behavioural depression and ataxia. The concentration of ethanol in the cage on the tenth day was lethal to naive mice within 8 hours.

The final blood levels of ethanol obtained in mice treated for ten days caused coma and eventually death in naive mice.

Ethanol administration for 10 days markedly increased the rate of elimination of ethanol from blood and also from the brain.

These observations are thought to provide evidence for both metabolic and pharmacological tolerance during the administration of ethanol in this way.

Evidence of dependence on ethanol was sought by close observation of the behaviour of mice after the ethanol concentration in the inspired air was brought down rapidly to near zero levels. This could be accomplished in approximately ten minutes. Within 15 min the mice began a period of often intense locomotor excitation. This reached a peak after about 75 min and declined thereafter, to be followed by a period of locomotor depression. Two hours after ethanol withdrawal, treated mice showed fine tremor and piloerection. A characteristic convulsion (Goldstein & Pal, 1971) could be elicited by holding a withdrawn mouse by the tail. These signs of withdrawal usually persisted until some 10 or 12 h after ethanol withdrawal. Objective evidence of some of these changes has been obtained using an Animex Activity Meter type S.

Goldstein & Pal described similar changes, but with a more protracted time course.

It is concluded that the administration of ethanol in this way fulfils the conditions for a valid model of ethanol dependence.

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REFERENCES

DURITZ, G. & TRUITT, E. B. Jr. (1964). A rapid method for the simultaneous determination of acetaldehyde and ethanol in blood using gas chromatography. *Quart. J. Stud. Alc.*, **25**, 498.

GOLDSTEIN, D. B. & PAL, N. (1971). Alcohol dependence produced in mice by inhalation of ethanol: grading the withdrawal reaction. *Science*, **172**, 288–290.

MYERS, R. D. & VEALE, W. L. (1971). The determinants of alcohol preference in animals. In *The Biology of Alcoholism*. ed. by B. Kissim and H. Begleiter. Vol. II Physiology and Behaviour. Plenum Publishing Corporation, New York.

A stain for the detection of choline acetyltransferase

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Evidence for the existence of choline acetyltransferase (EC 2.3.1.6.) (ChA) in multiple forms has been obtained by column isoelectric focusing (Malthe-Sørenssen & Fonnum, 1972). While the physiological significance of this observation remains to be clarified, the value of the method for characterizing ChA in different tissues is evident. We describe here a stain for ChA which, together with thin layer separative methods, allows rapid characterization of ChA from both central and peripheral tissues.

CoA produced in the reaction of ChA with acetyl-CoA and choline is localized, as it is formed, by reaction with oxoglutarate dehydrogenase (EC 1.2.4.2.) (Tubbs & Garland, 1969) in the presence of thiazolyl blue (3,(4,5-dimethylthiozalyl-2)-2)-2,5-diphenyl monotetrazolium bromide; MTT) and phenazine methosulphate (N-methylphenazonium methosulphate; PMS) (Pearse, 1957).

$$CoA + 2\text{-oxoglutarate} + NAD \rightleftharpoons succinyl-CoA + CO_2 + NADH_2$$

$$NADH_2 + MTT \xrightarrow{PMS} NAD + MTT$$

(oxidized form, yellow) (reduced form, blue precipitate)

2-Oxoglutarate, oxoglutarate dehydrogenase, NAD, MTT and PMS, together with acetyl-CoA and choline are layered onto the surface of the electrophoresis plate in a buffered, 1% agar gel (pH 7·0). A blue colouration coincident with ChA activity appears during subsequent incubation at 37° C (30–60 min).

We have used this method for locating ChA activities following separation by isoelectric focusing in Sephadex (G-75) thin layers (Radola, 1969). The results for crude extracts of goldfish skeletal muscle, squid head ganglia, guinea-pig and pigeon brains further demonstrate the heterogeneity of ChA.

REFERENCES

MALTHE-SØRENSSEN, B. & FONNUM, F. (1972). Multiple forms of choline acetyltransferase in several species demonstrated by isoelectric focusing. *Biochem. J.*, **127**, 229–236.

PEARSE, A. G. E. (1957). Intracellular localisation of dehydrogenase systems using monotetrazolium and metal chelation of their formazans. *J. Histochem. Cytochem.*, **5**, 515–527.

RADOLA, B. J. (1969). Thin-layer isoelectric focusing of proteins. *Biochim. Biophys. Acta*, **194**, 335–338.

TUBBS, P. K. & GARLAND, P. B. (1969). Assay of coenzyme A and some acyl derivatives. In *Methods in Enzymology, XIII*, 535–536.

Interaction of alcohol and drugs on psychomotor skills as demonstrated by a driving simulator

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Diazepam may enhance the alcohol induced impairment of human reactive and coordinative skills (Hughes, Forney & Richards, 1965 ; Linnoila & Mattila, 1972) whereas neuroleptics, in low doses, impair attention in particular (Linnoila & Mattila, 1972). The present study was conducted to confirm the validity of our simple laboratory tests (Häkkinen, 1958) as screening procedures for drug-alcohol interactions considered harmful for driving behaviour.

Ninety male drivers, aged 19–21 years, doing their compulsory military service in motorized troops, volunteered for the study. Every subject was carefully tested before entering the army. They were distributed in nine test groups 10 subjects in each. The drugs, given double blind in identical capsules, were: 10 mg of diazepam, 25 mg of codeine phosphate, or 750 mg of isoniazid. Drugs were administered in combination with placebo or similar alcoholic bitter drink, the amount of ethanol being 0·5 g/kg. The subjects were asked to assess their capacity of performance and the quality of their drug and drink.

The main simulator device was a Sim-L-Car with one point system shadow projection. Clutch, brake, gears, flashing lights and changes in steering and speed were recorded in a number of individual movements. The device also permitted recording of the lateral distance of the vehicle to objects in the path of the car. An eight-channel recorder registered continuously certain characteristics. Two sets of counters, 15 each, could be switched on in varying intervals. Pulse frequency and reaction time were also measured. The subjects and the screen were TV-filmed. The process of testing was programmed by punched cards. Emergency situations were simulated by a car suddenly entering from a side road. The driving period was 40 min starting 30 min after the drug intake.

Alcohol alone increased the collision frequency and made the subjects prone to ignore instructions and traffic rules. Diazepam alone increased the collision frequency and so did codeine. Diazepam given in combination with alcohol resulted in a further increase